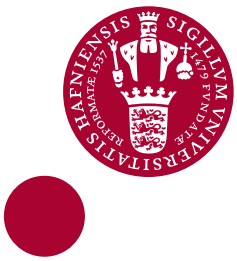

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Ancient individuals from the North American Northwest Coast reveal 10,000 years of regional genetic continuity

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Recent genomic studies of both ancient and modern indigenous people of the Americas have shed light on the demographic processes involved during the first peopling. The Pacific Northwest Coast proves an intriguing focus for these studies because of its association with coastal migration models and genetic ancestral patterns that are difficult to reconcile with modern DNA alone. Here, we report the low-coverage genome sequence of an ancient individual known as "Shuká Káa" ("Man Ahead of Us") recovered from the On Your Knees Cave (OYKC) in southeastern Alaska (archaeological site 49-PET-408). The human remains date to ∼10,300 calendar (cal) y B.P. We also analyze low-coverage genomes of three more recent individuals from the nearby coast of British Columbia dating from ∼6,075 to 1,750 cal y B.P. From the resulting time series of genetic data, we show that the Pacific Northwest Coast exhibits genetic continuity for at least the past 10,300 cal y B.P. We also infer that population structure existed in the late Pleistocene of North America with Shuká Káa on a different ancestral line compared with other North American individuals from the late Pleistocene or early Holocene (i.e., Anzick-1 and Kennewick Man). Despite regional shifts in mtDNA haplogroups, we conclude from individuals sampled through time that people of the northern Northwest Coast belong to an early genetic lineage that may stem from a late Pleistocene coastal migration into the Americas.

ancient DNA | paleogenomics | Native American | indigenous | peopling

The initial peopling of the Northwest Coast has received much attention because of its proximity to Beringia and associated implications for an initial coastal migration into the Americas (1–3). Genetic clues for the peopling of the Northwest Coast, however, may be obscured by later demographic events in the region. Studies based on mtDNA and Y-chromosomal markers suggest that populations in the region likely experienced admixture from other groups that entered the region after the initial peopling (4–6). Studies using genome-wide data (7–9) inferred ancient gene flow into North America likely stemming from subsequent movements after the initial settlement. However, because of the limited genomic data from populations in this geographic region, those studies leave questions regarding the degree of temporal genetic continuity of Northwest Coast populations.

In the Americas, the oldest thus far whole genome stems from Anzick-1, dating back to ∼12,600 calendar (cal) y B.P. and reportedly associated with Clovis technology (10, 11). Anzick-1 has proven to be surprising in a broader genetic sense, showing greater affinity with Central and South American groups than with Northern groups, despite the ancient burial existing in North America (but comparative indigenous populations from the United States are currently lacking). Shuká Káa, unearthed from On Your Knees Cave (Prince of Wales Island, AK), is not associated with Clovis culture but instead, is associated with a maritime tradition consistent with a coastal migration model and has been dated at ∼10,300 cal y B.P. (3). Shuká Káa exhibited the same mitochondrial haplogroup as Anzick-1 (12), suggesting a link in maternal lineage. Approximately 300 km southeast of the On Your Knees Cave archaeological site is Lucy Island off the

Significance

The peopling of the Americas has been examined on the continental level with the aid of SNP arrays, next generation sequencing, and advancements in ancient DNA, all of which have helped elucidate evolutionary histories. Regional paleogenomic studies, however, have received less attention and may reveal a more nuanced demographic history. We present genome-wide sequences of individuals from the northern Northwest Coast covering a timespan of ∼10,000 years and show that continental patterns of demography do not necessarily apply on the regional level. Compared with existing paleogenomic data, we show that geographically linked population samples from the Northwest Coast exhibit an early ancestral lineage and find that population structure existed among Native North American groups as early as the late Pleistocene.

Author contributions: J.L., B.M.K., M.D., and R.S.M. designed research; J.L., C.V., M.R., B.M.K., and R.S.M. performed research; J.L., E.W., B.M.K., M.D., and R.S.M. contributed new reagents/analytic tools; J.L., A.A., U.A.P., M.D., and R.S.M. analyzed data; D.A., E.J.D., T.E.F., and J.S.C. provided archaeological context; B.P., J.M., R.W., and T.E.F. organized community engagement; R.W. and E.J.D. interpreted results; and J.L., A.A., D.A., B.P., J.M., R.W., E.J.D., T.E.F., J.S.C., B.M.K., M.D., and R.S.M. wrote the paper.

The authors declare no conflict of interest.

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Data deposition: The sequences reported in this paper have been deposited in the NCBI Sequence Read Archive (BioProject ID: PRJNA356890).

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ANTHROPOLOGY

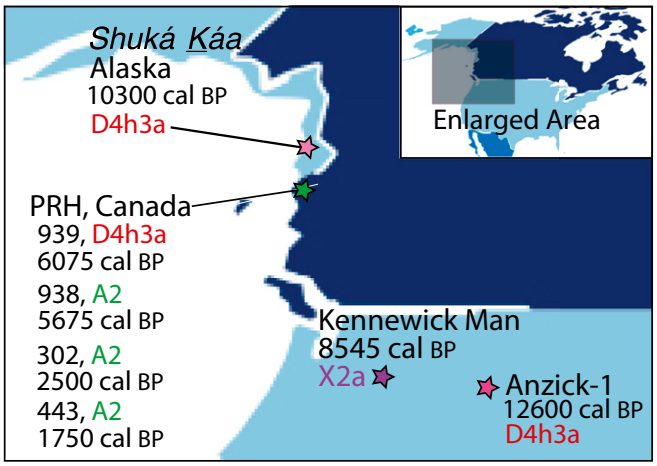

Fig. 1. Sampling locations of ancient samples and their associated mtDNA haplogroups.

coast of British Columbia, Canada. This island is the location of an individual, cataloged as 939, who died ~6,075 cal y B.P. (13). Individual 939 displays genetic affinity to Pacific Northwest coast groups, such as the Coast Tsimshian (henceforth Tsimshian), that currently live in the same region, but it is difficult to reject 939 as ancestral to both North and South American groups (9).

The only other ancient genome from North America is the Ancient One (also known as Kennewick Man), unearthed in the US state of Washington and dating back to ~8,545 cal y B.P. (14). Kennewick Man also displays surprising results as an early Holocene individual who resided in the Pacific Northwest. His mtDNA belongs to the northern North America limited haplogroup X2a, but his nuclear genome shows affinities with Central and South American populations, similar to patterns observed for Anzick-1. However, a direct ancestry test shows the greatest link to living individuals from the Confederated Tribes of the Colville Reservation, a Native population living in the same geographic region as Kennewick Man (14). On a broader scale, numerous areas of the Americas exhibit patterns consistent with genetic continuity of peoples in the same geographic region over time (9).

To test hypotheses related to different demographic scenarios for the peopling of the Northwest Coast, we generated a low-coverage genome (including the complete mitochondrial genome) for Shuká Káa from Alaska (Fig. 1 and Table 1). In addition, we generated two ancient low-coverage genomes, 302 and 443, from Prince Rupert Harbor (PRH), British Columbia (Fig. 1 and Table 1) dating to 2,500 and 1,750 cal y B.P., respectively. Along with previously described genomes from the Americas, we test two hypotheses about the peopling of the Northwest Coast. First, we test whether the people of this geographic region show temporal genetic continuity dating back to at least 10,300 cal y B.P. Second, we test whether the ancestors of the Northwest Coast experienced additional gene flow in the mid-Holocene to further explore the previously observed shift in mtDNA haplogroups on the Northwest Coast (13).

Community Engagement

It is important to note that the interactions between scientists and indigenous community members associated with this study were and continue to be respectful. Shuká Káa is the indigenous name given to the ancient individual found in On Your Knees Cave on Prince of Wales Island in southeast Alaska. His remains were identified in 1996, the same year in which the Ancient One was unearthed from the banks of the Columbia River near Kennewick, WA. However, unlike the antagonistic relationships that were to develop over the handling of the Ancient One's remains, T.E.F., a US Forest Service archaeologist, and other researchers engaged with Tlingit- and Haida-speaking communities in Alaska and developed strong working relationships with community members (details are in Datasets S1–S4). With appropriate tribal engagement and discussions, our analyses were conducted on some of the last remaining tissue subsampled from Shuká Káa's molars for DNA analysis before his repatriation to the Tlingit and reburial in his ancestral land.

Farther south, J.S.C. and R.S.M. established a partnership with the Metlakatla and Lax Kw'alaams First Nations in 2007 to aid in the study of the population histories of those communities. They are located in the PRH region of British Columbia. As part of the active partnership, J.S.C. and R.S.M. visit the communities on a regular basis to develop research studies and discuss interpretations of results as well as manuscripts written for peer review publication. The First Nations agreed to allow destructive DNA methods of samples of ancestral individuals analyzed in this study.

Results

A Mitochondrial Genome Reassessment. The individuals analyzed exhibit DNA damage patterns consistent with ancient DNA (Fig. S1). The complete mitochondrial genome of the Shuká Káa individual belongs to haplogroup D4h3a and was compared with 52 modern (available in GenBank) and 2 ancient D4h3 mitochondrial genomes (939 and Anzick-1) (11, 13). The resulting tree (Fig. S2) clearly shows that Anzick-1 is ancestral to the entire D4h3a clade, whereas the ancient Northwest Coast mitochondrial genomes belong to two different subbranches known as D4h3a9 and D4h3a12, with the latter here defined and encompassing a modern sample of an individual currently living in Bolivia (*SI Text*).

Today, the haplogroup D4h3a is virtually absent in northern North America. To the contrary, the mitochondrial genomes of the more recent ancients from the Northwest Coast (443 and 302) are classified as A2 (Table 1), the most commonly reported mitochondrial haplogroup of native North America. Thus, based on the mtDNA data alone, it might be plausible that the native people of the northern Northwest Coast experienced a drastic change in their mtDNA gene pool in a rather short period, possibly because of additional gene flow in the mid-Holocene (mitochondrial genome change hypothesis). However, considering that the mtDNA haplogroup frequencies are likely to change

Table 1. Sequencing results and whole-genome coverage

Sample	Age ^{14}C B.P.	Age (cal y B.P.)	mtDNA haplogroup	Sex*	Sequencing strategy	Libraries sequenced	Mean read depth	Whole-genome coverage
Shuká Káa	9,200 ± 50	10,344 ± 83	D4h3a	M	Genome enrichment	8	2.85	0.059
443	1,820 ± 55	1,750 ± 70	A2d	M	Genome enrichment	2	4.01	0.560
302	2,440 ± 75	2,498 ± 142	A2p	F	Genome enrichment	2	4.74	0.701
939[†]	5,710 ± 40	6,075 ± 185	D4h3a7	F	see Raghavan et al. (9)	see Raghavan et al. (9)	5.15	0.343

F, female; M, male.

*Sex was determined from sequence reads using the method described by Skoglund et al. (27).

[†]Previously described by Raghavan et al. (9). Cal year conversion from radiocarbon dates was achieved with the method described in Cui et al. (13).

radically because of drift in small populations over time (15), such a mitochondrial genome discontinuity might simply be the result of the limited number of complete mitochondrial genomes analyzed from the area, particularly from ancient individuals (16). Thus, because the mitochondrial genome can describe only part of the ancestral genetic history of the Northwest Coast, we extended the analyses to the entire genome to test alternative hypotheses.

Autosomal Genome Assessment. We used outgroup f_3 statistics to assess the shared ancestry among the ancient individuals and 169 worldwide populations (9). Outgroup f_3 statistics of a worldwide dataset show that all four ancient individuals (Shuká Káa, 939, 443, and 302) display greater affinity with Native American groups than with other worldwide populations (Fig. 2*A* and Fig. S3). Ranked outgroup f_3 statistics suggest that 939, 443, and 302 tend to share greatest affinity with Northwest Coast groups, whereas Shuká Káa ostensibly shows closer affinity to groups farther south (Fig. S4). However, because of the low coverage of the Shuká Káa sample, the relationship is not statistically significant.

To further elucidate the relationship among the ancient individuals of the Northwest Coast and their relationship to modern populations, we examined maximum likelihood trees created with TreeMix (17). C/T and G/A polymorphic sites were removed from the dataset to guard against the most common forms of postmortem DNA damage (18). We observe that 302 and 443 form a sister clade to the modern Tsimshian (masked for European ancestry) (Fig. S5 *A* and *B*, respectively). Individual 939 is an outgroup to both North and South Americans (Fig. S5*C*) as is Shuká Káa (Fig. 3*B*). However, adding a migration event introduces an edge connecting Europeans and Shuká Káa, which leads to Shuká Káa forming a clade with the Tsimshian and Athabascan (Fig. S6). The signal may represent Native American dual ancestry (19) or be a result of possible contamination (Table S1).

Principal components analysis reveals a tight clustering of 939, 443, and 302, which also overlap with modern North American indigenous populations (Fig. 2*C* and Fig. S7). Shuká Káa falls in close proximity but overlaps with both North and South American groups. The admixture clustering analysis shows a more complicated pattern,

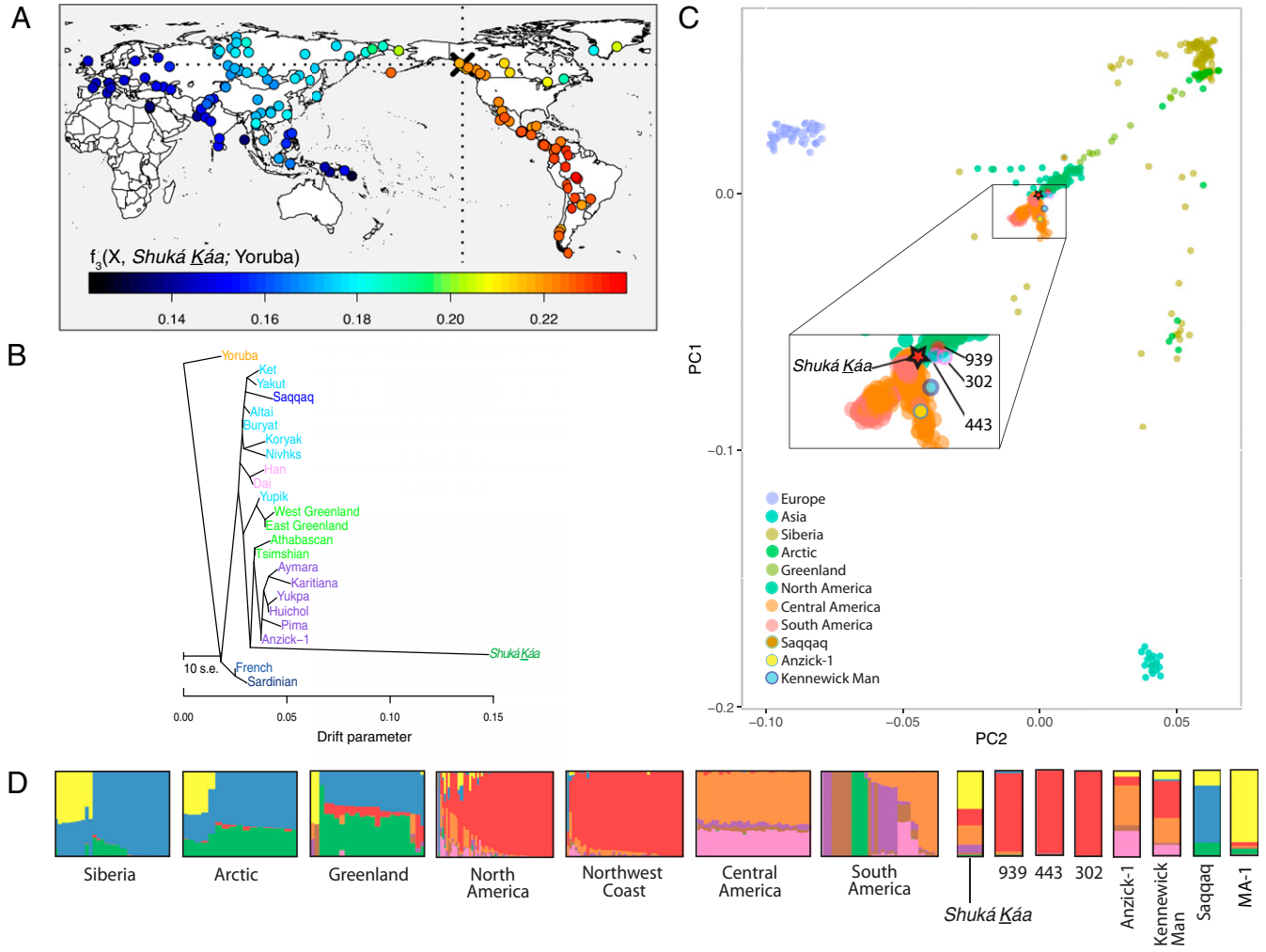

Fig. 2. Genetic affinity of Shuká Káa and the other Northwest Coast prehistoric humans to global and regional indigenous populations. (*A*) Heat map represents the outgroup f_3 statistics estimating the amount of shared genetic drift between Shuká Káa and each of 156 contemporary populations since their divergence with the African Yoruban population. (*B*) Maximum likelihood tree generated by TreeMix using whole-genome sequencing data from Raghavan et al. (9) and with the Tsimshian genome masked for European ancestry. (*C*) Principal components analysis projecting Shuká Káa, 939, 302, 443, Anzick-1 (11), Saqqaq (30), and Kennewick Man (14) onto a set of non-African populations from Raghavan et al. (9), with Native American populations masked for nonnative ancestry. (*D*) Cluster analysis generated by ADMIXTURE for a set of indigenous populations from the Americas, Siberia, the Arctic, and Greenland and the Anzick-1, Kennewick, MA-1 (19), Saqqaq, Shuká Káa, 939, 302, and 443 samples. The number of displayed clusters is $K = 8$, which was found to have the best predictive accuracy given the lowest cross-validation index value.

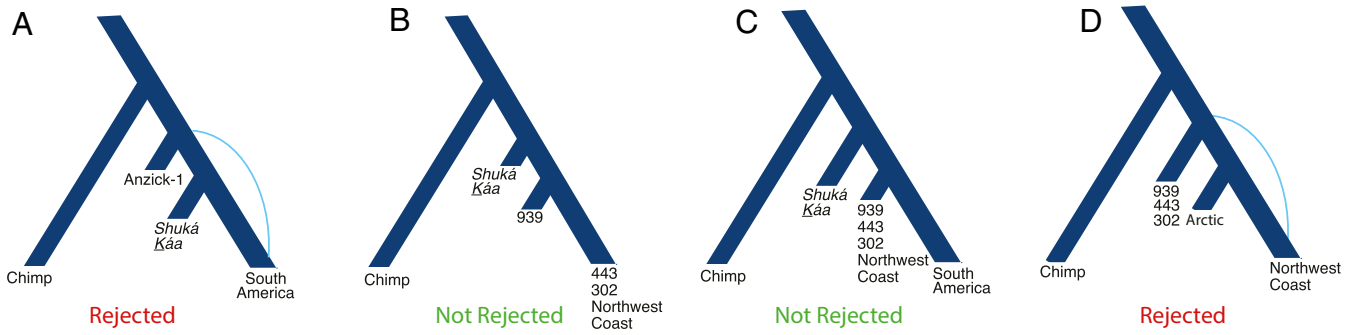

Fig. 3. Hypothetical scenarios for the regional peopling of the Northwest Coast. (*A*) Scenario tested by the *D* statistic where Anzick-1 is basal to both Shuká Káa and South America, which is rejected, indicating a closer affinity to South America. (*B*) Scenario tested by the *D* statistic where Shuká Káa is basal to 939 and both contemporary and ancient Northwest Coast individuals, which is not rejected. (*C*) Scenario tested by the *D* statistic where Shuká Káa is basal to ancient and modern Northwest Coast and South America, which is not rejected. (*D*) Scenario tested by the *D* statistic where 939, 443, and 302 are basal to the Arctic (Yup'ik and Inuit) and contemporary Northwest Coast populations, which is rejected, indicating a closer affinity to contemporary Northwest Coast populations. Supporting *D* statistics are listed in Table S2.

where Shuká Káa exhibits mainly components identified in North American and Siberian/Arctic individuals as well as smaller fractions found in South American populations (Fig. 2*D*). However, individuals 939, 302, and 443 all exhibit a major component found in North American populations.

To further test the hypothesis that people of the Northwest Coast exhibit a close genetic relationship with ancient individuals from the same region, we used the *D* statistic (20). Given the TreeMix admixture results between Shuká Káa and European populations, we performed a contamination correction to the *D* statistic as described in the work by Raghavan et al. (19) using observed *D* statistics with European populations (*SI Text*). Hypothetical scenarios based on the *D* statistics are depicted in Fig. 3. The *D* statistic does not support a scenario of genetic continuity between Anzick-1 and Shuká Káa with respect to South Americans (Fig. 3*A* and Table S2). The relationship of Shuká Káa, however, is more complex when examined with specific North American ancient and modern groups. Comparing Shuká Káa with 939 and the more recent ancient individuals from the Northwest Coast (443 and 302), we cannot reject an equally diverged relationship with respect to Shuká Káa (Fig. 3*B* and Table S2). However, we see the same relationship when the comparison is performed between both ancient and modern Northwest Coast individuals and individuals from South America, where Shuká Káa is basal to both groups (Fig. 3*C* and Table S2). Individual 939 displays a similar pattern with TreeMix and *D* statistics (Fig. S5*C* and Table S2), where the individual seems basal to both the Northwest Coast and South America. However, the admixture results show a predominately "North American" component, and contamination-corrected *D* statistics for 939 indicate a significant relationship toward the Northwest Coast (Table S2, tests 17 and 18).

The *D* statistic did not reveal a signal of gene flow between Arctic populations (Inuit and Yup'ik) and either the modern or ancient Northwest Coast populations compared with Shuká Káa (Table S2, tests 19–28). However, comparing the more recent ancient individuals, the tree was rejected with 939, 302, and 443, indicating greater affinity toward the Northwest Coast populations than the Arctic (Fig. 3*D* and Table S2).

Because certain *D* (Table S2, tests 8 and 9) and f_3 (Fig. S3*D*) statistics yielded nonsignificant results, we next wanted to examine whether the basal relationship that Shuká Káa exhibited to Northwest Coast and South American populations could be caused by its age relative to the split time of those groups. To address this hypothesis, we simulated genetic data with FastSimCoal2 (21), which allowed us to sample Shuká Káa 10,300 y in the past (*SI Text*). We considered one scenario (scenario 1), in which Shuká Káa is on the branch leading to the Northwest Coast, and another scenario (scenario 2), in which the sample was on a branch that diverged earlier than the split of the Northwest Coast and South American populations (Fig. S8*A*). Results for 1,000 simulated replicates under each scenario are plotted in Fig. S8*B*, indicating that only a small fraction of simulated replicates from scenario 1 could reject the null hypothesis that Shuká Káa is equally diverged to the Northwest Coast and South American lineages, although the simulations placed Shuká Káa on the Northwest Coast branch. However, the reason for this lack of power may be because of the amount of data (which we controlled to yield a similar number of *D* statistic informative sites as the empirical data). We, therefore, also considered a set of simulations where we increased the expected number of *D*-statistic informative sites by an order of magnitude. Results from these simulations (Fig. S8*C*) show that the clear majority of simulated replicates from scenario 1 could reject the null hypothesis that Shuká Káa is equally diverged to the Northwest Coast and South American lineages, with Shuká Káa having higher affinity to the Northwest Coast. Furthermore, results

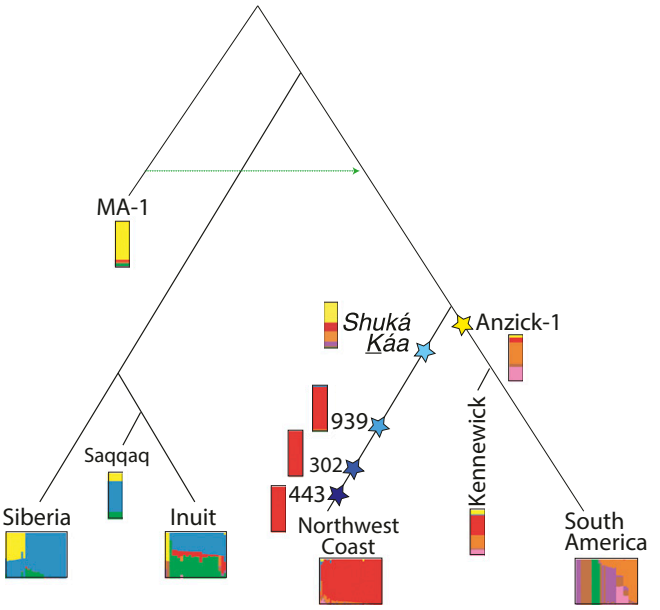

Fig. 4. Shuká Káa in relation to other Native American groups. Schematic showing Shuká Káa placed on the branch leading to North Americans, which is supported by simulation-based *D* statistics.

for scenario 2 indicate that the null hypothesis is generally not rejected as expected from the simulated scenario (Fig. S8 *B* and *C*).

We next considered models that examine a less direct relationship between Shuká Káa and the more recent ancient individuals in addition to the spread of the D4h3a mtDNA haplogroup across the Americas. Because Anzick-1 from Montana predates Shuká Káa (~12,600 vs. ~10,300 y B.P.) and shares the same mtDNA haplogroup, we performed *D* statistics to assess their relationship. The tree with Shuká Káa and 939 as sisters relative to Anzick-1 is not rejected (Fig. S9*A*). The trees with modern Northwest Coast (mtDNA haplogroup A2) as sister to either 443 (mtDNA haplogroup A2) or 302 (mtDNA haplogroup A2) relative to 939 (mtDNA haplogroup D4h3a) are also not rejected (Fig. S9*B*). These results are inconsistent with a change in the overall gene pool of the Northwest Coast after the early Holocene, although it led to the observation of very different mitochondrial haplogroups detected over time in the region.

We also explored the relationship of Shuká Káa to Kennewick Man (14), who was unearthed in Washington and dates to ~8,545 cal y B.P. Although Kennewick Man does not share the same mtDNA haplogroup with Shuká Káa, they coexisted within 1,700 y of each other (about 68 generations). In all tests with the *D* statistic, Shuká Káa displayed a basal relationship to both ancient and modern Northwest Coast populations with respect to Kennewick Man (Fig. S9*C* and Table S2).

Discussion

These data support a shared ancestry for the indigenous peoples of the Northwest Coast dating back to at least ~10,300 cal y B.P. The individual supporting this scenario is Shuká Káa, who belongs to mtDNA haplogroup D4h3a. Although both Anzick-1 and 939 also belong to this mtDNA haplogroup, later ancient and modern individuals of the Northwest Coast do not (13, 22). Despite belonging to different mtDNA haplogroups, Shuká Káa exhibits a close nuclear DNA relationship with 302 (~2,500 cal y B.P.) and 443 (~1,750 cal y B.P.), both of whom belong to mtDNA haplogroup A2, which is observed today at high frequencies among modern Northwest Coast populations (22). Thus, based on the limited mtDNA data alone, the hypothesis of a change in the genetic composition of the Northwest Coast appearing by the time of 302 and 443 might be plausible. However, our more extended genome-wide analysis does not support a change in the genetic composition of the Northwest Coast appearing by the time of 302 and 443, both of whom form a sister clade with the Tsimshian (Fig. S5). Instead, we observe a trend of genetic continuity through time, which is exemplified by individual 939 who displays affinities with both the more recent Northwest Coast ancient individuals and Shuká Káa. Individual 939 shares the same mtDNA haplogroup D4h3a as Shuká Káa while belonging to the predominant North American ancestry component observed in 443 and 302 (Fig. 2*D*). Furthermore, previously published complete mitochondrial genome data from individual 938 (13), who is similar in age and found on the same island site as 939, exhibit an A2 mtDNA haplogroup. These results indicate that the two haplogroups were already present in the ancestral population and are not the result of later gene flow into the area.

Also of interest is the evidence for the placement of Shuká Káa on different lineages than Anzick-1 and Kennewick Man (Fig. S9*D*). Despite their shared North American geographies and time periods as well as evidence that a single peopling event occurred in the late Pleistocene (23), our analyses show that population structure existed in the late Pleistocene of North America. These data are concordant with the late Pleistocene archaeological record documenting distinct contemporaneous archaeological cultures in different regions of North America (24).

We find that the placement of Shuká Káa (based on *D*-statistic simulations) is consistent with residing on a branch with modern day indigenous people from the Northwest Coast, whereas Anzick-1 fits separately on a branch that leads to the southern lineage, including populations from Central and South America (Fig. 4). Our result suggests that Shuká Káa was part of a population closely related to the ancestors that gave rise to the current populations of the northern Northwest Coast.

Table 2. Ancient overlapping sites used for autosomal-based analyses

Ancient individual	f_3	Principal components analysis and ADMIXTURE	TreeMix
Shuká Káa	245 (minimum); 1,906 (median); 1,914 (maximum)	2,327	1,044
302	7,656 (minimum); 56,400 (median); 56,500 (maximum)	59,205	29,592
443	9,881 (minimum); 75,000 (median); 75,170 (maximum)	79,233	35,620

On a broader scope, ADMIXTURE analysis revealed a component that dominates contemporary North American populations, which increases over time regarding the Northwest Coast (Fig. 2*D* and Fig. S10). We also observe a small fraction of this component in the 24,000-y-old MA-1 individual from Siberia (19) (Fig. 2*D*). This result suggests that the North American component reflects an early ancestral lineage that has drifted through time to high frequency on the Northwest Coast.

It should be noted that, because of the highly degraded nature of the DNA extracted from Shuká Káa sample, the autosomal analyses had less than the optimal number of overlapping sites (Table 2). This outcome is likely the cause for the lack of significance in both the ranked f_3 and *D* statistics when regarding Shuká Káa. In light of these observations, projections that consider missing data and contamination corrections were used to help mitigate the low number of sites available (*SI Text*). Despite these limitations, the analyses presented here, taken as a whole and compared with higher-depth samples, paint a sensible and convincing picture of the peopling of the Northwest Coast.

We conclude that the Northwest Coast exhibits an ancestral lineage that stems from the initial peopling of the region. The observed temporal change of mtDNA haplogroups in the area was probably caused by sampling, and no clear signs of gene flow into the area after the first settlement have been identified. Shuká Káa, who lived some 10,300 y ago, was part of an ancestral population that may have first populated the region but was distinct from ancestral populations related to Anzick-1. Although we cannot use our data to identify the specific ancestral location of this coastal lineage, the facts that Shuká Káa lived over 10,000 y ago and that Anzick-1 is related to the South American lineage suggest that the ancestral population likely existed north of the continental ice before the deglaciation of the interior corridor sometime after 12,600 y B.P. (23). This inference is supported by both the ADMIXTURE analysis showing a large component of ancestry of Shuká Káa shared with Siberian populations (the yellow component) and previous analysis by Verdu et al. (25) that suggests a closer relationship to Northeast Asian groups by Pacific Northwest populations than Central and South American populations.

The collaborative approach of this study shows an example of how indigenous community members and scientific researchers can work together in a positive and mutually beneficial way. In addition, the results presented here reveal the power of regional studies to elucidate demographic complexities, shedding light on the peopling of the Northwest Coast and the early ancestral lineages of North America.

Methods

DNA Extraction and Library Preparation. We used standard ancient DNA extraction methods following stringent guidelines to work with ancient human remains and conducted these in dedicated ancient DNA laboratories. DNA was extracted from molars belonging to individuals Shuká Kåa, 302, and 443. Furthermore, each DNA extract was converted into Illumina libraries (*SI Text*).

Genome Enrichment. We captured from eight libraries of Shuká Kåa DNA with the MyBaits whole-genome enrichment kit enhanced with protocol modifications recommended for the study of ancient DNA (*SI Text*). The eight captured libraries from each individual were pooled and sequenced (single end) on four lanes of an Illumina HiSeq 2000 run. Two captured libraries from 302 and 443 were pooled and run on two additional lanes.

Contamination Estimates. Contamination estimates using the mitochondrial genome were run on all three samples using the Scmutzi program described by Renaud et al. (26). The method jointly estimates present day human contamination and reconstructs the endogenous mitochondrial genome by considering both deamination patterns and fragment length distributions. Because Shuká Kåa and 443 were typed as male using the method described by Skoglund et al. (27), contamination based on the X chromosomes was also performed for these samples using the method described by Korneliussen et al. (28) and applied through the ANGSD software suite (www.popgen.dk/angsd/index.php/ANGSD). The Shuká Kåa sample did not have sufficient coverage along the X chromosome to perform the estimate.

f_3 And *D* Statistics. To test the genetic affinity of the ancient individuals with global populations, we performed f_3 outgroup statistics using the method outlined by Patterson et al. (20). We also examined the genetic affinities of each individual and various populations using ranked f_3 statistics, which are shown in Fig. S4. To examine the relationship between the ancient individuals (Shuká Kåa, 939, 443, 302, and Anzick-1), we performed an ABBA-BABA test or *D* statistic (29) using the definition used by ANGSD (28). The chimpanzee genome was used as an outgroup sequence. The tests also included the whole genome of a contemporary Tsimshian (9), which was masked for European ancestry, and all comparisons with Shuká Kåa used a correction for European contamination as did several comparisons with 939 (*SI Text*). To guard against potential bias from DNA damage in the ancient individuals, transitions were not considered during the tests.

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